



Nitrogen deposition and decreased precipitation does not change total nitrogen uptake in a temperate forest

Mingxin Zhou^{a,1}, Guoyong Yan^{a,b,1}, Yajuan Xing^{b,c,1}, Fei Chen^a, Xin Zhang^b, Jianyu Wang^a, Junhui Zhang^d, Guanhua Dai^d, Xingbo Zheng^d, Wenjing Sun^a, Qinggui Wang^{a,b,*}, Tong Liu^{a,**}

^a School of Forestry, Northeast Forestry University, 26 Hexing Road, Harbin 150040, China

^b College of Agricultural Resource and Environment, Heilongjiang University, 74 Xuefu Road, Harbin 150080, China

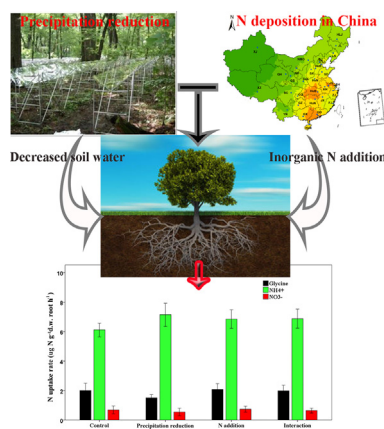
^c Institute of Forestry Science of Heilongjiang Province, 134 Haping Road, Harbin 150081, China

^d Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang 110016, China

HIGHLIGHTS

- Trees showed a strong preference for NH_4^+ uptake over glycine and NO_3^- uptake.
- Plasticity in root traits use allowed trees to acclimation to environmental change.
- The change of root physiology may be the key to the increase of NH_4^+ uptake.
- The total amount of plant N uptake did not change after long-time treatments.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 8 July 2018

Received in revised form 11 September 2018

Accepted 12 September 2018

Available online 13 September 2018

Editor: Elena PAOLETTI

Keywords:

Nitrogen uptake

Decreased precipitation

Nitrogen addition

Root plasticity

Temperate forest

ABSTRACT

Decreased precipitation and increased anthropogenic by derived nitrogen (N) are important climate change factors that alter the availability of soil water and N which are crucial to root function and morphological traits. However, these factors are seldom explored in forests. To clarify how altered precipitation and N addition affect the uptake of organic and inorganic N by fine roots, a field hydroponic experiment using brief ^{15}N exposures was conducted in a temperate forest in northern China. The root traits related to nutrient foraging (root morphology and mycorrhizal colonization) were measured simultaneously. Our results showed that all three tree species preferred ammonium (NH_4^+) over glycine and nitrate (NO_3^-), and NH_4^+ contributed 73% to the total N uptake from the soil. Uptake of glycine was higher than that of NO_3^- . Decreased precipitation, N addition, and their interaction increased NH_4^+ uptake rate compared with the control. Decreased precipitation decreased the glycine and NO_3^- uptake rate. Moreover, N addition, decreased precipitation and their interaction changed root morphological traits and significantly decreased mycorrhizal colonization. Although our treatments resulted in changes to the root traits and the forms of N uptake by plants, the total amount of N uptake did not change among all treatments.

* Correspondence to: Q. Wang, College of Agricultural Resource and Environment, Heilongjiang University, 74 Xuefu Road, Harbin 150080, China.

** Corresponding author.

E-mail addresses: qgwang1970@163.com (Q. Wang), 66476673@qq.com (T. Liu).

¹ These authors contributed equally to this work.

We conclude that although fine root traits of dominant tree species in temperate forests have high plasticity in response to climate change, nutrient balance in plants causes the total amount of N uptake to remain unchanged.
© 2018 Elsevier B.V. All rights reserved.

1. Introduction

Nitrogen (N) is a fundamental element regulating plant growth and development in terrestrial ecosystems, specifically in temperate and boreal forests, where N is generally considered as a major limiting factor for plant growth and development (Elser et al., 2007; LeBauer and Treseder, 2008). There are various N forms present in the soil, e.g., NH_4^+ and NO_3^- and organic N forms such as various amino acids (Nordin et al., 2001; Persson and Näsholm, 2001; Liu et al., 2017). Therefore, plants uptake a wide array of N forms, which is regarded as an acclimation to reduce limitations (Näsholm et al., 2009). More recently, the importance of plant strategies that use different forms of N has been increasingly acknowledged. Previous studies suggested that plant species may display various mechanisms to optimize the acquisition of N forms due to the complexity of plant N strategies (Näsholm et al., 2009). As a result, uptake patterns of inorganic and organic N may differ in various plant species and ecosystems (Inselsbacher and Näsholm, 2012; Liu et al., 2017). For example, plant species in the same ecosystem can avoid inter-species competition (competition theory) through the use of different N forms (McKane et al., 2002; Miller et al., 2007). Moreover, organic N in temperate forests is high due to low mineralization rates of soil organic matter, and but the contribution of organic N to plant N demands is inconsistent in different studies (Leduc and Rothstein, 2010; Liu et al., 2017). Therefore, a comprehensive understanding of various N forms contribution to plant N demands in various tree species of temperate forest remains incomplete.

In addition to ecosystem types and plant species, soil resource conditions also play an important role in regulating N uptake by plants (Xu et al., 2011). In recent decades, anthropogenically derived N and changes in precipitation regimes have been altered the availability of soil N and water resources that are crucial to plant performance and N uptake strategies (Larsen et al., 2011). Anthropogenic N deposition has led to an increase in soil N availability (Liu et al., 2013), which can alter uptake of various N forms and specific root traits to achieve effective N uptake (Lilleskov et al., 2002; Li et al., 2015). For example, N addition decreased the length, biomass and mycorrhizal colonization rates of the fine roots to regulate nutrient uptake (Li et al., 2015; Wang et al., 2017). Moreover, the quantity and forms of soil available N are very sensitive to reduced soil water availability (Gholamhoseini et al., 2013). Decreased water availability reduces soil N mineralization and N availability (Emmett et al., 2004; Sanaullah et al., 2012). Additionally, acquisition of N by living roots or mycorrhizal fungi is a complex process involving transport in soil via flow and diffusion. Decreased water availability can reduce/change the delivery of various N forms to the root surface through water flow due to the difference in the mobility of different N forms, which may effects on the uptake of various N forms (Buljovic and Engels, 2001). For example, under drought conditions, plants become more reliant on inorganic N due to greater mobility (e.g., NO_3^- and NH_4^+) as an N source because it is more mobile than organic N (e.g., amino acids) (Tobar et al., 1994; Hawkins et al., 2000). The availability of water also affects root morphology, affecting uptake of N by roots (Kadam et al., 2017). However, this process is seldom explored in forests.

Atmosphere N deposition and decreased precipitation are altering the environment. Plant species can adjust to environmental changes through plasticity of root physiological and morphological traits. Under N additions and precipitation decreases, plasticity in N uptake might therefore be an adaptive strategy giving plants a competitive advantage associated with root tissue construction and maintenance, because they can meet their N demands with any N forms occurring in their root surface (Gutschick, 1981; Grime, 2006). Similarly, since future climate change and regional anthropogenic activity will involve changes in N deposition

and precipitation patterns, to understand the possible interactions between different climate and anthropogenic drivers on soil N availability and plant N limitations becomes extra important. However, currently, few previous studies have examined the effects of N additions, precipitation decrease and their interactions on the quantity and forms of plant N absorption and the root traits related to N uptake simultaneously.

To clarify how the plasticity of the uptake of N forms in response to N deposition, decreased precipitation and their interaction in a temperate forest, we conducted an experiment with an N addition, a 30% of decreased precipitation and their interaction to quantify their effects on the uptake strategies for different N forms using field hydroponic experiments and root traits measurement in an old broad-leaved Korean pine (*Pinus koraiensis*) mixed forest in northern China. N addition increased soil inorganic N availability (Liu et al., 2013), we expected that inorganic N more likely to reach the root surface. Therefore, we hypothesize that N addition, will increase inorganic N (NH_4^+ and NO_3^-) uptake. Moreover, decreased precipitation decreased mass flow or diffusivity of organic and inorganic N (Hawkins et al., 2000), we hypothesize that decreased precipitation will decrease inorganic N uptake. Plants developed a suite of effective strategies for root acquiring nutrients and water from the soil by depending on specific root traits (Ostonen et al., 2017). Thus we also hypothesize that N addition, decreased precipitation and their interaction will increase the length of absorption root to increase the rate of root N uptake. Generally, our results provide an important opportunity to understand how the changes in precipitation patterns and anthropogenic alterations of N cycles affect root N uptake strategies.

2. Materials and methods

2.1. Site description and experimental treatments

The experimental sites are located in the Changbai Mountains Natural Reserve in Jilin Province, northeastern China (42°24'N, 128°06'E, and 738 m a.s.l.). This region belongs to a typical temperate-continental climate zone. The long-term (1982–2003) mean annual air temperature is 3.6 °C, with a mean growing season (from May to October) temperature of 15 °C, a mean winter season temperature of −0.6 °C, the highest monthly mean temperature of 21.5 °C occurring in August and the lowest monthly mean temperature of −17.3 °C occurring in January (Forest Ecosystem Research Station of Changbai Mountains, Chinese Academy of Science). Mean annual precipitation is approximately 790 mm, of which 630 mm occurs between May and August. The soil in the study sites was developed from volcanic ash and is classified as an Eutric Cambisol (FAO classification); the bulk density was 0.35 g cm^{−3} in the surface soil (0–10 cm) and 0.68 g cm^{−3} in the deep soil layer (10–20 cm); the contents of total carbon (C), N, phosphorus (P) and organic matter in the 0–20 cm soil layer were 156.6 g C kg^{−1}, 7.17 g N kg^{−1}, 12.20 g P kg^{−1}, 270.00 g kg^{−1}, respectively (Wang et al., 2012). The vegetation type is an old broad-leaved Korean pine mixed forest that is over 300 years old. The dominant tree species are *Pinus koraiensis* (PKS), *Tilia amurensis* (TAR), *Fraxinus mandshurica* (FMR) and *Quercus mongolica*. The mean canopy height and diameter at 1.3 m breast height for these three species were 26 m and 34.2 cm, respectively (Zheng et al., 2017). The tree density on the site was 432 trees hm^{−2}. The main shrub species include *Philadelphus schrenkii*, *Euonymus alatus*, *Lonicera japonica*, *Corylus mandshurica*, and *Deutzia scabra*, and the main herbaceous species include *Anemone raddeana*, *A. cathayensis*, *Cyperus microiria*, *Funaria officinalis*, *Adonis vernalis*, *Brachybotrys paridiformis*, and *Filipendula palmata*. The aboveground tree litterfall is 4.23 Mg hm^{−2}, and aboveground productivity is 9.46 Mg hm^{−2}.

In this study, six 50×50 m plots surrounded by a >20 m wide buffer strip were randomly established in an old broad-leaved Korean pine mixed forest at Changbai Mountains in May 2009. Three plots were treated with decreased precipitation, and the other three plots were set as ambient treatment. The precipitation removal facility (Fig. S1) was installed using high light transmittance (transparency 95%) polycarbonate V-shaped translucent panels to intercept through-fall, the polycarbonate panels covering 30% of the plot area intercepted approximately 30% of the natural through-fall in the growing season, and the panels were removed to allow snow to fall onto the forest floor in winter. A previous study suggested that precipitation amounts have decreased in north China over the last 50 years (Wang et al., 2006). In the drought years of 1985, 1997, 1999, 2001, and 2003, precipitation rate decreased approximately 200 mm, which is approximately 30% less than the long-term mean annual precipitation of 740 mm in the Changbai Mountains area in the last 30 years (Chinese Ecosystem Research Net (CERN)). To ensure normal air flow, panels were fixed on an aluminum frame at approximately 1 m above the soil surface. To measure the effect of the N addition on root N uptake strategies, each of these six plots was split into two 25×50 m subplots using a stainless steel sheet (to avoid bringing into other ions) inserted 50 cm deep into the ground to avoid nutrient mobilization between each adjacent subplot. One of the subgroups of each plot received additional N while the other did not. Based on previous study data, in the Changbai Mountains area, the mean annual atmosphere deposition of N is approximately $23 \text{ kg N hm}^{-2} \text{ yr}^{-1}$ (Lü and Tian, 2007). Thus, the selected level of N addition for the study was $50 \text{ kg N hm}^{-2} \text{ yr}^{-1}$, which was approximately two times the historic N deposition rate for the area. In each N addition subplot, ammonium nitrate (NH_4NO_3) was weighed, mixed with 40 L of deionized water, and sprayed monthly onto the forest floor with a backpack sprayer over the entire growing season (May to October), beginning from May 2009 (it has lasted for 8 years). Two passes were made across each subplot to ensure an even distribution of N fertilizer. The control plots simultaneously supplied 40 L of deionized water without N addition to avoid a difference in the moisture application. Finally, this process created four treatments (each one with three replicates): the control treatment (CK), N addition (+N, $50 \text{ kg N hm}^{-2} \text{ yr}^{-1}$), decreased precipitation treatment (−W, 30% of through-fall, approximate $200 \text{ mm} \cdot \text{yr}^{-1}$) and the combined N addition and decreased precipitation treatment (+N − W).

2.2. Field hydroponic experimental design and fine root excavation

We selected PKS (*Pinaceae*), FMR (*Oleaceae*) and TAR (*Tiliaceae*), which are dominant and mature tree species in this old growth forest ecosystem, to examine their N uptake by roots. To examine root N uptake, a mixture solution of NH_4^+ , NO_3^- , and glycine was used. NH_4^+ and NO_3^- are sources of plant inorganic N, and glycine is a plant organic N source. As glycine is one of the dominant and low-molecular weight amino acids in most soils, it is often used in experiments related to plant organic N uptake (Lipson and Näsholm, 2001; Svennerstam et al., 2008; Liu et al., 2017). Each solution contained equal parts of NH_4^+ , NO_3^- , and glycine ($33 \mu\text{mol N L}^{-1}$ for each N form), which provided an equal chance for roots to choose among the N forms. There were four treatments (three labeling solutions and one control solution) with three replicates for each plant species in each subplot. Each labeling solution only contained one of the three N forms that were ^{15}N labeled (99 atom% ^{15}N -glycine, 10 atom% ^{15}N - KNO_3 or 10 atom% $(^{15}\text{NH}_4)_2\text{SO}_4$), while the other two were applied at their natural abundances. The control solution contained all three N forms in an unlabeled form to examine natural variation in $\delta^{15}\text{N}$ values among the species in each subplot. These operations allowed the discerning of N uptake strategies for the different chemical forms of N among the species and treatments. In each solution, ampicillin (10 mg L^{-1}) was included to decrease microbial activity and avoid decomposition of the amino

acid, and 0.2 mmol CaCl_2 was also included to maintain the function and integrity of the roots (Warren and Adams, 2007).

The labeling experiments were performed in August 2017. All labeling solutions had been aerated before being used. Three trees in each species with similar size were randomly selected in each subplot. Then, four pairs of fine roots of each tree with a diameter <2 mm were dug out diagonally in four directions (one pair for each direction) (Liu et al., 2017). Each pair of roots in the same direction was washed with deionized water, and then the root pair was immersed in the labeling solution in 15 ml centrifuge tube. Thus each treatment had six replicates for each species in each subplot. After 2 h, the fine roots were excised using scissors and rinsed with 50 mmol KCl solution for 3 min and then washed with deionized water to remove the ^{15}N remaining on the surface of the fine roots. Each fine root collected was dried at 70°C for 48 h to measure dry mass. Dried roots were grounded into a fine powder, and placed into tin capsules for measurements of N content and $^{15}\text{N}/^{14}\text{N}$ ratios using an isotope ratio mass spectrometer (Isoprime 100, Isoprime Ltd.) coupled to an automatic, online elemental analyzer (Vario ISOTOPE cube). However, for each individual N uptake was measured in 4 directions, this data has been pooled for each tree.

Soil samples were collected immediately following in situ labeling experiments from beneath each tree in four directions. Three soil cores (diameter 2 cm) were taken from the top layer (0–10 cm) of each direction by using a handheld auger, and twelve soil cores of each tree from four directions were pooled into one composite sample. All soil samples were immediately brought to the laboratory, sieved to 2 mm and stored at 4°C until measurements were taken. Soil NH_4^+ and NO_3^- were extracted from 10 g fresh soil using $0.05 \text{ mol/L K}_2\text{SO}_4$ extraction procedures, and were determined by using an autoanalyzer (AA3, Bran-Luebbe, Hamburg, Germany). The soil total N (TN) was measured by using a multi N/C 3100 analyzer (multi N/C 3100, Analytik Jena AG, Germany). The soil total organic N (TON) was equal to the TN minus the inorganic N (NH_4^+ and NO_3^-). The soil pH was measured using a pH meter (Sartorius PB-10, Gottingen, Germany) with a 1:2.5 (soil: water, dry weight/volume) ratio of soil to water then shaking for 30 min. Soil samples were dried for 48 h at 105°C to assess soil moisture.

Intact segments of the fine root system (including root order 1–3, shorter-lived absorptive root based on McCormack et al. (2015)) were very carefully excavated following the end of in situ labeling experiments and the collection of soil samples from beneath each tree in four directions. A random location was chosen in each direction under each tree in each plot. A shovel was used to collect soil monoliths measuring $20 \times 10 \times 10$ deep in each site, and the intact root segments were collected from each soil monoliths. Because the roots were taken around each tree species in each plot, it was easy to identify the roots of each species based on branching pattern, color, and texture of the root bark or epidermis. Root samples were extracted from monoliths by washing with deionized water to remove soil particles, then immediately placed in a cooler with ice and transported to the laboratory minutes to a few hours. Individual roots were dissected with forceps by order, following the procedure described in Fittera and stickland (1992). Then, root samples of each species were scanned using an Expression 10000XL 1.0 scanner (Epson Telford Ltd., Telford, UK). The mean diameter, length and surface area of root tips were measured with the root system analyzer software (WinRhizo, 2012b, Regent Instruments, Inc., Québec, Canada). For root mycorrhizal colonization analyses, 30 root order 1 of each tree species were sampled in each plot. The number of root order 1 colonized by fungi was viewed and determined under a dissecting microscope based on color and fungal mantle, hyphae and rhizomorph texture. The mycorrhizal colonization was calculated as: mycorrhizal colonization (%) = mycorrhizal root tips $\times 100$ / total root tips.

2.3. Calculations and statistical analysis

^{15}N atom percent excess (APE) was calculated based on the difference in atom percent ^{15}N between ^{15}N -labeled and control roots. The

net uptake rates of N for each plant species ($\mu\text{g N g}^{-1} \text{ d.w. root h}^{-1}$) were calculated by multiplying N content ($\text{mg N g}^{-1} \text{ d.w.}$), APE and dry root biomass. The net N uptake rate was expressed as the following based on Liu et al. (2017):

$$\text{Net N uptake rates} = \frac{\text{N Content (ug/g)} \times \frac{\text{APE}}{100}}{\text{Time(h)} \times \frac{\text{at}\%^{15}\text{N tracer}}{100}}$$

Then, contributions of each N form were calculated by dividing the N uptake rate by the sum of uptake rates of the three forms.

The normal distribution of all the data was tested by the Kolmogorov-Smirnov test, and the homogeneity of variances was tested by Levene's test. The effects of N addition, decreased precipitation and their interaction on the individual root length, individual root surface and mean root diameter of different root orders, mycorrhizal colonization, and the mean N uptake rates of each N form for all tree species was analyzed by one-way analysis of variance (ANOVA) with Tukey-Kramer HSD. A multi-factor analysis of variance was used to examine the effects of the treatments, types of tree, and N forms on N uptake rates. Correlations between soil properties and N uptake rates were analyzed by Pearson correlation. The effects of the treatments and species on soil properties were analyzed using a two-way analysis of variance. Statistical calculations were performed using the SPSS software version 17.0 (SPSS, IBM, USA). Figures were designed using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA). Statistically significant differences were accepted at $p < 0.05$.

3. Results

3.1. Effects of N addition and decreased precipitation on soil property

Soil organic N, inorganic N (NH_4^+ and NO_3^-), pH and soil moisture are presented in Table 1. TON did not have significant differences among all treatments. Soil NH_4^+ concentrations under decreased precipitation were significantly higher than those in the control ($p < 0.05$). The interaction of N addition and decreased precipitation significantly increased

NO_3^- concentrations compared with the effects of the control ($p < 0.05$). N addition significantly decreased pH value, decreased precipitation significantly reduced soil moisture, and their interaction significantly decreased pH value and soil moisture compared with the control ($p < 0.05$). Moreover, the microenvironments of different tree types may also affect soil properties. Tree types and interaction of N addition and tree types had a significant effect on TON and NH_4^+ . The interaction of N addition, decreased precipitation and tree types had a significant effect on TON (decrease), NH_4^+ (increase), and soil moisture (decrease).

3.2. Effects of N addition and decreased precipitation on uptake rates of different N forms

All tree species absorbed NH_4^+ at the highest rate and absorbed NO_3^- at the lowest rate among all treatments (Fig. 1). For same tree species under different treatments (Fig. 1A), FMR in N addition and interaction of N addition and decreased precipitation showed significantly high uptake rates of glycine compared with that in the control. The NH_4^+ uptake in interaction of N addition and decreased precipitation was not significantly different from the other treatments ($p < 0.05$). For PKS, N addition, decreased precipitation and interaction of N addition and decreased precipitation significantly decreased uptake rates of glycine compared with the uptake rates under the control. Decreased precipitation significantly increased uptake rates of NH_4^+ compared with uptake rates under the other treatments, and uptake rates of NO_3^- were significantly lower under decreased precipitation and interaction of N addition and decreased precipitation than that under the N addition and control ($p < 0.05$). For TAR, uptake rates of glycine were significantly higher under the interaction of N addition and decreased precipitation than under decreased precipitation. The interaction of N addition and decreased precipitation significantly increased uptake rates of NH_4^+ compared with those rates under the other treatments. N addition and interaction of N addition and decreased precipitation resulted in significantly high uptake rates of NO_3^- compared with the uptake rates of NO_3^- under decreased precipitation and the control ($p < 0.05$). For the different treatments and combined tree species (Fig. 1B), N addition, decreased precipitation, and the interaction of N addition decreased precipitation significantly increased uptake rates of NH_4^+ compared with

Table 1
Soil physicochemical property in different tree types under different N treatments in August 2017.

Treatments	Species	TON (mg/g)	NH_4^+ (mg/g)	NO_3^- (mg/g)	pH	W (%)
Control	FMR	4.57 ± 0.02b	0.229 ± 0.021a	0.003 ± 0.001a	5.33 ± 0.57a	51.46 ± 0.07a
	PKS	4.60 ± 0.04ab	0.203 ± 0.034a	0.003 ± 0.001a	5.05 ± 0.12a	53.33 ± 5.39a
	TAR	4.63 ± 0.03a	0.168 ± 0.026b	0.003 ± 0.001a	5.02 ± 0.30a	52.34 ± 3.56a
	Mean	4.60 ± 0.03A	0.200 ± 0.031B	0.003 ± 0.000B	5.13 ± 0.33A	52.38 ± 5.39A
−W	FMR	4.59 ± 0.04ab	0.207 ± 0.042a	0.003 ± 0.001a	5.44 ± 0.30a	46.96 ± 6.52a
	PKS	4.55 ± 0.05b	0.252 ± 0.044a	0.002 ± 0.001a	5.37 ± 0.32a	43.82 ± 4.35b
	TAR	4.62 ± 0.01a	0.186 ± 0.013b	0.001 ± 0.001b	5.10 ± 0.25a	47.73 ± 5.56a
	Mean	4.59 ± 0.03A	0.215 ± 0.033A	0.002 ± 0.001B	5.30 ± 0.29A	46.17 ± 5.48B
+N	FMR	4.60 ± 0.03a	0.205 ± 0.031b	0.003 ± 0.001a	5.11 ± 0.11a	54.02 ± 5.80a
	PKS	4.59 ± 0.01a	0.215 ± 0.011a	0.003 ± 0.002a	4.94 ± 0.56a	54.59 ± 11.68a
	TAR	4.60 ± 0.01a	0.205 ± 0.010b	0.003 ± 0.002a	4.85 ± 0.23a	54.14 ± 11.57a
	Mean	4.60 ± 0.01A	0.208 ± 0.006B	0.003 ± 0.002B	4.96 ± 0.30B	54.25 ± 9.69A
−W + N	FMR	4.55 ± 0.03b	0.245 ± 0.033a	0.007 ± 0.002a	5.10 ± 0.63a	47.45 ± 8.43a
	PKS	4.60 ± 0.05a	0.204 ± 0.044b	0.005 ± 0.002b	4.83 ± 0.42a	40.48 ± 6.90b
	TAR	4.60 ± 0.03a	0.204 ± 0.033b	0.004 ± 0.002b	4.77 ± 0.26a	45.26 ± 3.33a
	Mean	4.58 ± 0.03A	0.217 ± 0.024A	0.005 ± 0.002A	4.90 ± 0.44B	44.40 ± 6.22B
−W	NS	NS	NS	NS	NS	NS
+N	NS	NS	NS	**	NS	NS
Tree types	**	**	NS	NS	NS	NS
−W* + N	NS	NS	***	NS	NS	NS
−W* Tree types	NS	NS	NS	NS	NS	NS
+N* Tree types	*	*	NS	NS	NS	NS
−W* + N* Tree types	**	**	NS	NS	NS	**

Each value is the Mean (SE) of nine replications, and statistical analysis unit is each plots. ANOVA: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS not significant. Different capital letters represent significant differences among different treatments. Different Lowercase letters represent significant differences among different tree types in same treatment. −W represents the decreased in precipitation treatments; +N represents the N addition treatments; +N − W represents the N addition and decreased in precipitation treatments; W represents the soil moisture content; TON represents the soil total organic nitrogen; FMR represents the *Fraxinus mandshurica*; PKS represents the *Pinus koraiensis*; TAR represents the *Tilia amurensis*.

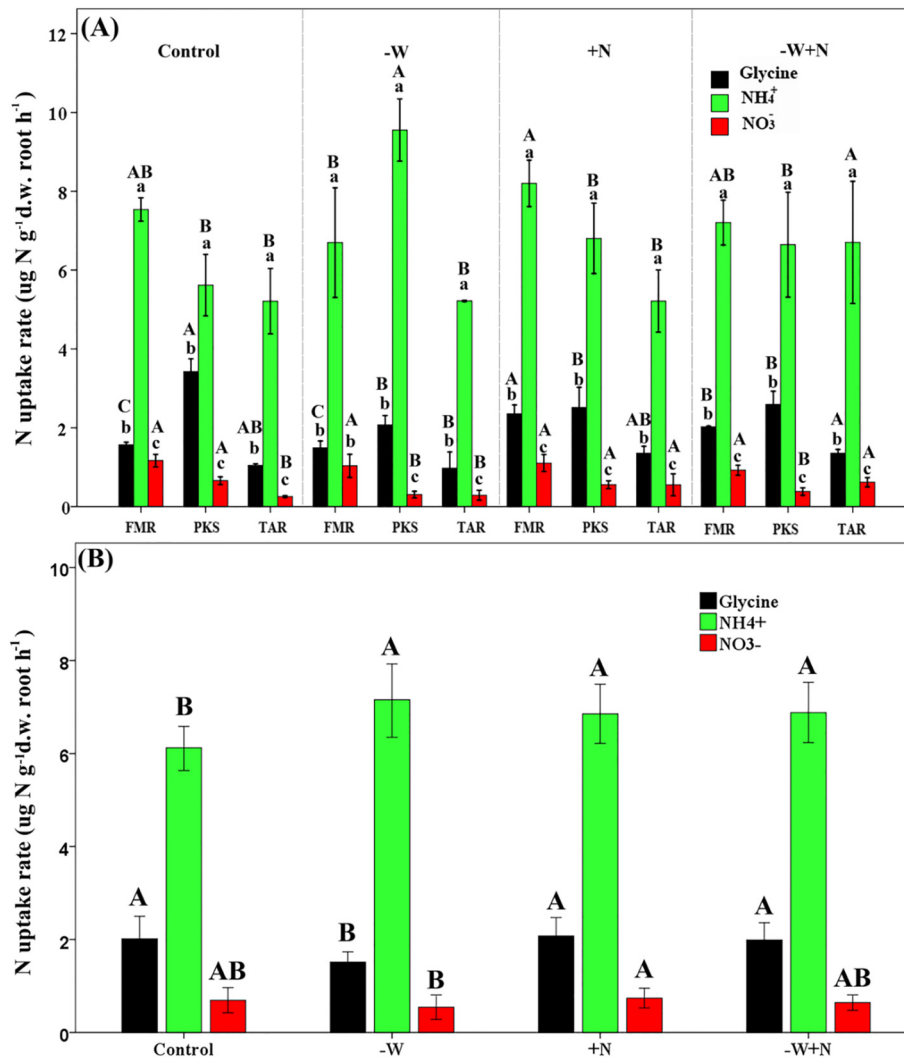


Fig. 1. (A) Uptake rates of glycine, NO₃⁻ and NH₄⁺ by three dominant tree species in a temperate forest under different treatments, and (B) total uptake rates of glycine, NO₃⁻ and NH₄⁺ under different treatments. Values are presented as means ± SE of nine replicates. (A and B) Different lowercase letters indicate significant differences in uptake rates among N forms for each species (one-way ANOVA, $p < 0.05$). (A and B) Different uppercase letters above the bars indicate significant differences in uptake rates between different treatments for same species and same N form (one-way ANOVA, $p < 0.05$). FMR represents the *Fraxinus mandshurica*; PKS represents the *Pinus koraiensis*; TAR represents the *Tilia amurensis*. -W represents the decreased in precipitation treatments; +N represents the N addition treatments; +N - W represents the N addition and decreased in precipitation treatments.

the NH₄⁺ uptake rates under the control ($p < 0.001$). Furthermore, decreased precipitation significantly decreased uptake rates of glycine compared with those rates under other treatments, and uptake rates of NO₃⁻ were also significantly lower under decreased precipitation than under N addition ($p < 0.001$).

Although all treatments did not change the order of uptake preference for the three N forms among all species, they did change the contributions of different N forms (Fig. 2). For different treatments, uptake of NH₄⁺ accounted for 70% of TN uptake (sum of the uptake of the three N forms) in the control and was significantly lower than that in N addition and decreased precipitation (74% and 77%, respectively; Fig. 2, $p < 0.05$). The contribution of glycine uptake under decreased precipitation was approximately 16% of the total uptake of all three N forms and was significantly lower than that in the other treatments ($p < 0.05$). For different species, the contribution of NH₄⁺ to TAR was 77% of the TN uptake, which was significantly higher than that to FMR (69%, Fig. 2, $p < 0.05$). The contributions of glycine to TAR and PKS were 17% and 18% of the TN uptake respectively and were significantly lower than that to FMR (26%, Fig. 2, $p < 0.05$). The contribution of NO₃⁻ uptake to all three tree species was estimated at approximately 7% of the total uptake of the three N forms, with the highest value for PKS. However, when tested across all three N forms, the effects of N addition, decreased

precipitation and their interaction were not significant on the uptake rates of N, but tree types, N forms, and their interaction had significant effects on N uptake rates (Table S1). Moreover, these interaction terms among all treatments and tree types or all treatments and N forms had significant effects on the uptake rates of N (Table S1). All the treatments did not change the total amount of N uptake (Fig. 3).

3.3. Effects of N addition and decreased precipitation on root morphological traits and mycorrhizal colonization

N addition, decreased precipitation and their interaction significantly increased the individual root length, individual root surface area and mean root diameter of FMR among all root order (Fig. 4). N addition significantly increased individual root length, individual root surface area and mean root diameter in order 1 of PKS, while decreased these traits in orders 2–3 (Fig. 4). The individual root length and mean root surface area in orders 2 of PKS and TAR significantly increased with decreased precipitation, but not in orders 1 and 3 (Fig. 4). Moreover, individual root length, individual root surface area and mean root diameter increased significantly with interaction of N addition and decreased precipitation among all root order of TAR compared with the control. The individual root length, individual root surface

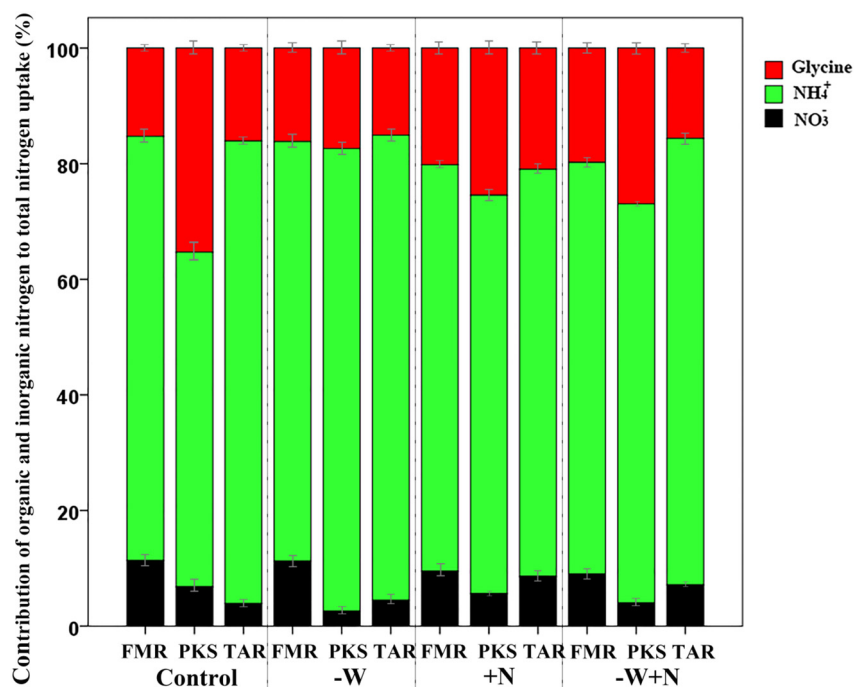


Fig. 2. The contribution of glycine, NO_3^- and NH_4^+ to total N uptake by 3 dominant tree species under different treatments in a temperate forest. Values are presented as means $\pm$ SE of three replicates. FMR represents the *Fraxinus mandshurica*; PKS represents the *Pinus koraiensis*; TAR represents the *Tilia amurensis*. -W represents the decreased in precipitation treatments; +N represents the N addition treatments; +N - W represents the N addition and decreased in precipitation treatments.

area and mean root diameter of the third order roots significantly increased with N addition in TAR. Across all species, N addition, decreased precipitation and their interaction significantly decreased the mycorrhizal colonization (TAR in decreased precipitation is the only exception) (Fig. 5).

3.4. Correlations between soil parameters and mean N uptake rates and contribution

Additionally, soil moisture was significantly negatively correlated with the uptake of glycine, and soil NH_4^+ concentrations were

significantly positively correlated with NH_4^+ uptake. Soil TON concentrations were significantly negatively correlated with NH_4^+ uptake (Table 2). There was no significant relationship between the pH value and all N absorption rates. Soil NO_3^- had no significant correlation with NO_3^- uptake. NO_3^- uptake did not correlate with any parameter. We also found positive correlation between NH_4^+ contribution and soil moisture, and the negative correlation between glycine contribution and soil moisture.

4. Discussion

4.1. Uptake rates of different N forms by dominant tree species

Our experimental results demonstrated that all labeled trees showed a strong preference for NH_4^+ uptake over glycine and NO_3^- uptake for all three N forms when presented in equal concentrations, providing support and confirmation for the hypothesis of Liu et al. (2017). The dominant plant species prefer the N source that is more available in their areas (McKane et al., 2002; Boudsocq et al., 2012; Li et al., 2015). Soil NH_4^+ is the dominant N source in temperate forests, which suggests that temperate forest trees may prefer NH_4^+ uptake (Liu et al., 2017). Many previous studies have shown that uptake of organic N could exceed that of inorganic N in some boreal and temperate forests, which is mainly caused by low temperatures that lead to low mineralization rates in soil (Bardgett et al., 2003; Leduc and Rothstein, 2010; Averill and Finzi, 2011). Our study does not support previous studies on temperate forests with a cold climate (Warren, 2009), but was similar to a study in a temperate forest in China (Liu et al., 2017). The reason for this may be that although the concentration of organic N was high in the temperate forest, a large proportion of this organic N probably becomes unavailable to plants as a result of adsorption processes and uptake by soil microbial biomass (Lipson and Näsaholm, 2001). Otherwise, under N-limited conditions it would be advantageous to use this organic N, and if there are costs associated with maintaining the cellular machinery to use a variety of organic N types (e.g., amino acid transporters) then under high N conditions this strategy may not be advantageous (Lilleskov et al., 2002). Based on an economics theory (Eissenstat and

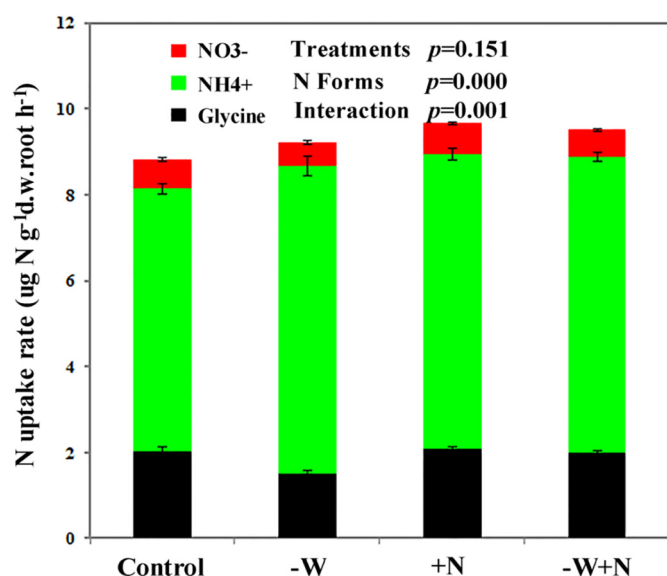


Fig. 3. Effects of N addition and decreased precipitation on the total N uptake change in a temperate forest (the mean of the 3 species). -W represents the decreased in precipitation treatments; +N represents the N addition treatments; +N - W represents the N addition and decreased in precipitation treatments.

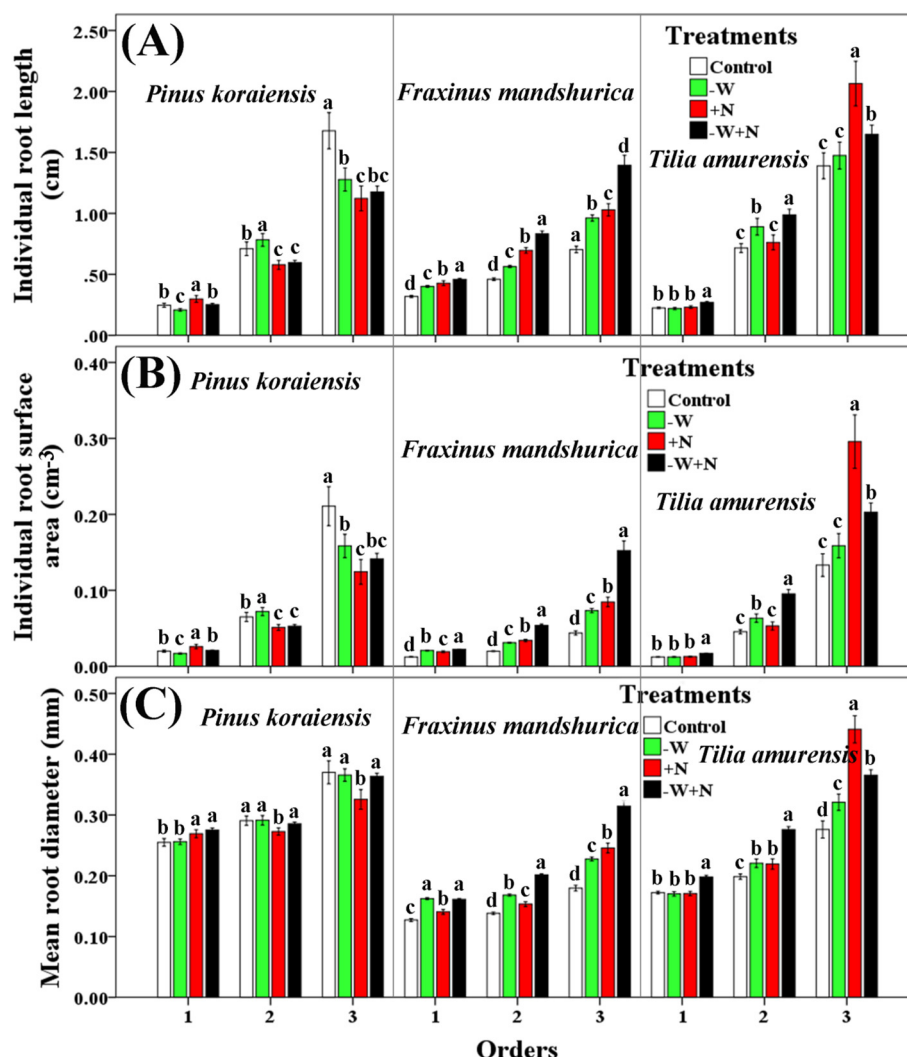


Fig. 4. Individual root length (A), individual root surface (B) and mean root diameter of different root orders (orders 1–3) under different treatments in three temperate tree species. Error bars represent 1SE of the mean. Significant differences ($p < 0.05$) between treatments are indicated by different lower-case letters. –W represents the decreased in precipitation treatments; +N represents the N addition treatments; –W + N represents the N addition and decreased in precipitation treatments.

Yanai, 1997), we speculated that because of the complexity and high consumption of organic N (such as amino acids) absorption and metabolism (Näsholm et al., 2009), it may lead plants to preferentially absorb inorganic N until when inorganic N cannot continuously supply plant needs. In addition, preferential uptake of NH_4^+ may also relate to the NH_4^+ dominance of this soil solution (Kou et al., 2015), and the

concentration of NH_4^+ at the study site was higher than glycine and NO_3^- . In this study, the 3 temperate tree species showed a preference for NH_4^+ over other N forms, and NO_3^- uptake rates were the lowest (Fig. 1A).

The contribution of NO_3^- uptake was only about 6–10% of total uptake of all inorganic N forms in this study. There may be three reasons

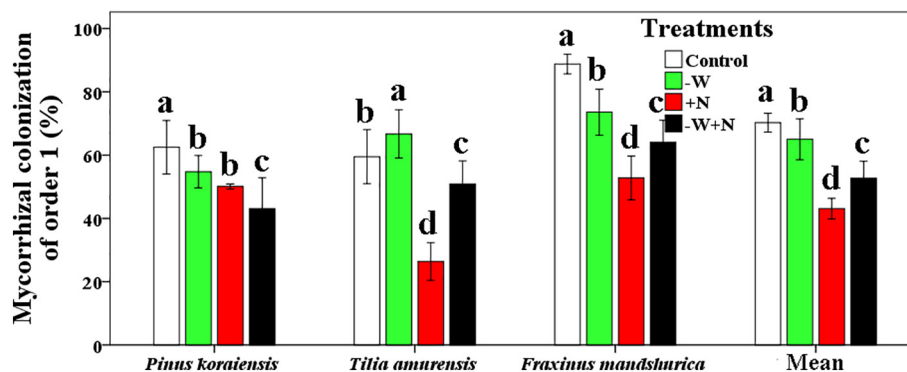


Fig. 5. Effect of different treatments on mycorrhizal colonization of root order 1 in three temperate tree species. The extreme right-hand mean is mean of the indices in the three tree species. Error bars represent 1SE of the mean. Different lower case letters within the clusters of bars indicate significant differences ($p < 0.05$). –W represents the decreased in precipitation treatments; +N represents the N addition treatments; –W + N represents the N addition and decreased in precipitation treatments.

Table 2

Correlation analysis between soil physicochemical property and N uptake rates and contributions. *p* values for significant effects and interactions are reported at *p* < 0.05 (*) level. Number in bold indicates that the effect was significantly different. W represents the soil moisture content; TON represents the soil total organic nitrogen.

	N uptake rate			N uptake contribution		
	Glycine	NO ₃ ⁻	NH ₄ ⁺	Glycine	NO ₃ ⁻	NH ₄ ⁺
pH	-0.18	0.002	0.11	-0.19	0.05	-0.18
W	-0.29	-0.21	0.28	-0.36*	-0.20	0.37*
NO ₃ ⁻	-0.08	0.13	-0.03	0.05	0.09	-0.23
NH ₄ ⁺	0.17	0.25	0.37*	-0.02	0.23	-0.12
TON	-0.17	-0.24	-0.36*	0.02	-0.22	0.13

for the slow absorption rate of NO₃⁻. First, although the labeling solution has been aerated before the root incubation, hypoxia would probably have been achieved during the 2 h of root incubation due to the high respiratory rates of root tips. The decrease of ATP production by oxidative phosphorylation strongly affects the NO₃⁻ uptake that relies on secondary active transport (Glass, 2009). Thus, under hypoxia, NO₃⁻ uptake would decrease more than that of NH₄⁺, which could explain the low contribution of NO₃⁻ uptake observed in the study. Second, under anaerobic conditions, denitrifiers may cause NO₃⁻ degradation concentration reduction, resulting in NO₃⁻ absorption decline (Zarnetske et al., 2012). Because of the addition of antimicrobial agents in the labeling solution, the second reason may have less effect on NO₃⁻ uptake. Third, the temperatures may affect NO₃⁻ uptake. Hydrosolution experiments showed that uptake of NH₄⁺ was favored by warm temperatures and NO₃⁻ by cool temperatures (Warren, 2009). Since in the present study data was sampled in the summer, thus the absorption rate of NO₃⁻ was very low. Furthermore, NH₄⁺ and amino acid inhibit the uptake of nitrate (Näsholm et al., 2009), the limitations and implications of Hydroponic experiment (using a solution that contains the three N compounds) may also lead to a decrease in NO₃⁻ uptake.

The absorption pattern of NO₃⁻ was relatively similar to that of glycine. Previous study suggested that glycine uptake was less temperature sensitive than uptake of NO₃⁻ (Raab et al., 1996). Therefore, the low glycine uptake could be associated to the relatively high temperatures in the study periods. However, due to the long-term acclimation of plant roots due to in situ environmental factors (Purugganan and Fuller, 2009), we deem that the deviation in the uptake of different N forms in short-term hydroponic experiments from naturally occurring levels is limited. Therefore, among all labeled trees, the relative contribution of different N forms in the temperate forest should be in the following order: NH₄⁺ > glycine > NO₃⁻.

4.2. Effects of N addition on uptake rates of different N forms

Although uptake rates of different measured N forms cannot be directly transferred to in situ uptake rates, they can accurately reflect their responses to environmental changes, such as N deposition and decreased precipitation. Eutrophication of terrestrial ecosystems, resulting from fertilizer additions or anthropogenic N deposition, occurs mainly through inputs of inorganic N forms (i.e., NH₄⁺ and NO₃⁻) (Galloway et al., 2008). We expected that in forests exposed to higher rates of inorganic N addition, uptake of N would shift towards NH₄⁺ and NO₃⁻ uptake and that organic N uptake would constitute only a smaller proportion of this processes. Surprisingly, long-term N addition with NH₄NO₃ resulted in higher uptake rates of only NH₄⁺, while no such trend was observed for the uptake of NO₃⁻ (Fig. 1B). The increase of NH₄⁺ uptake rates under N addition may not be related to the change of root morphology but may be related to root physiology. We speculated that prolonged N addition may induce an increase in the expression of NH₄⁺ transporters, resulting in increased absorption of NH₄⁺. Although we have not done any research on transporters of inorganic and organic N in the study, we think this may be a direction for future research.

Moreover, there are several possible mechanisms that may explain this effect of N addition on the uptake rates of soil NO₃⁻. First, microbial competition may be a mechanism for the effect to N addition on the uptake rates of NO₃⁻, as NO₃⁻ additions stimulated soil denitrification which could eventually lead to a decrease in the soil availability of NO₃⁻ resources (Gundersen, 1991; Geng et al., 2017). Second, the physical properties of NO₃⁻ may provide another mechanism. Soil NO₃⁻ is more mobile than NH₄⁺ and can easily leach out of the root-zone as it is not electrostatically bound, which would decrease the uptake of NO₃⁻ caused by the rapid decrease in NO₃⁻ from run-off (Arora and Srivastava, 2013). The third mechanism is plant physiology. NO₃⁻ uptake rates might be underestimated due to fast xylem loading and transport of NO₃⁻ to aboveground tissues (Liu et al., 2017). In this study, although we did not investigate these mechanisms, we found NO₃⁻ remained at similar concentrations between the control and N addition, which might indirectly suggest that the later steps in the N mineralization pathway (such as nitrification) may not limit production of available N, and unchanged soil NO₃⁻ uptake may be consistent with the second and third mechanisms mentioned above (Schimel and Bennett, 2004; Inselsbacher and Näsholm, 2012; Liu et al., 2017).

In addition to soil NO₃⁻ uptake, there was also an unexpected effect of N addition on the uptake rates of soil glycine. The long-term inorganic N addition with NH₄NO₃ did not result in lower uptake rates of glycine, illustrating the potential importance of organic N as N sources for plants and mycorrhizal fungi in high inorganic N environments (Inselsbacher and Näsholm, 2012). However, the mycorrhizal colonization significantly decreased with N addition but did not alter the rate of glycine uptake, suggesting that the mycorrhizal colonization does not affect glycine uptake. The glycine arriving at the membrane surfaces of non-mycorrhizal roots will be absorbed at rates comparable to that of mycorrhizal hyphae. This result is supported by previous study (Inselsbacher and Näsholm, 2012). Although N addition changed the different forms of N absorption rate, the root morphology traits and mycorrhizal colonization, did not change the total N absorption amount among all tree species, which indicated that N in plants had internal homeostasis under effects of N addition. Furthermore, our study indicates that the change of fine root morphological traits may make up for the effect of decreased mycorrhizal colonization on plants nutrient uptake under N addition.

4.3. Effects of decreased precipitation on uptake rates of different N forms

A previous study showed that uptakes of soil inorganic and organic N were also affected by decreases in soil moisture (Buljovic and Engels, 2001). A reduction in soil moisture may reduce microbial activity, net N mineralization, N diffusivity and mass flow, reducing available pools of inorganic N in soil (Borken and Matzner, 2009; Rouphael et al., 2012). Therefore, the decreased N uptake caused by changes in soil physical and biological processes under decreased precipitation may be expected (Rouphael et al., 2012). In this study, decreased precipitation significantly decreased the uptake of glycine, while the uptake of NO₃⁻ was unchanged, but decreased precipitation significantly increased the uptake of NH₄⁺ compared with the control (Fig. 1B). The result partly confirms our first hypothesis. Furthermore, the concentrations of organic N decreased, and concentrations of NH₄⁺ significantly increased in decreased precipitation compared with that in the control, suggesting that the uptake of organic and inorganic N may become coupled with concentration of organic and inorganic N in the soil. Based on our results, we present several directions for interpreting the possible mechanisms of how decreased precipitation may affect the uptake of organic and inorganic N. First, we expected that decreased precipitation should lead to organic N accumulation in the soil due to reduction in plant uptake. Nevertheless, there were no differences in TON across treatments in the study (Table 1), illustrating that competition for organic N between microbes and trees might increase under decreased precipitation. We speculated that soil microorganisms were

the superior competitors for N and that subsequently soil organic N might rapidly mineralize to NH_4^+ and NO_3^- in the soil microbial community at our site, which might lead to increase in uptake of NH_4^+ . Second, decreases in soil moisture may decrease mass flow or diffusivity of organic N, thereby restricting glycine transport from soil to root surfaces, and thus glycine uptake. This negative effect on N transport would be stronger for organic N than for inorganic N because diffusivity of organic N in the soil is more sensitive to soil moisture than that of inorganic N (Hawkins et al., 2000). Nevertheless, as noted in the methods, the N-uptake rate has been measured in short-term studies by incubating the roots in a nutrient solution. This method ensured the maintenance of a nearly constant concentration of N over the entire surface of the root system during the uptake experiment. It is obvious that the rate of N uptake under these conditions was dependent mainly on root physiological characteristics rather than flow or diffusivity ability of N forms (Bassirirad, 2000). Thus, changes in inorganic transporters expression are also noteworthy under decreased precipitation. Third, we speculated that plants may spend relatively more C assimilates on root growth to improve water and N uptake under decreased precipitation. The individual root length and individual root surface increased with decreased precipitation, which supported our speculation. Nevertheless, the mycorrhizal colonization significantly decreased with decreased precipitation. This may be because that acclimation of tree species to drier conditions may have selected in root systems, thereby facilitating soil exploration and nutrient absorption with less symbiotic aid (Comas et al., 2012; Valverde-Barrantes et al., 2015). However, the reduction in the uptake of glycine and increase in NH_4^+ uptake may become more balanced in long-term plant acclimation, thereby causing no effect on tree total N demand under decreased precipitation, which is consistent with our result (Fig. 3). Our results suggest that trees under low soil moisture stress may have flexibly adapted to new conditions and established a new equilibrium through adjusting their root growth, morphology, and physiochemical characteristics between plant growth and plant N uptake.

5. Conclusion

Plants may acquire N from a wide range of N forms, including inorganic N forms such as NH_4^+ and NO_3^- and organic N forms such as various amino acids. In this study, we showed that all labeled trees demonstrated a strong preference for NH_4^+ uptake over glycine and NO_3^- uptake from all three N forms when presented in equal concentrations in temperate forest soils, suggesting that NH_4^+ -N forms play a critical role in the N nutrition of plants in this biome. Uptake of inorganic and organic N was responsive to long-term N addition and decreased precipitation, all treatments had a positive effect on NH_4^+ uptake, and decreased precipitation had a negative effect on the uptake of glycine, implying that NH_4^+ may be the primary N sources in soil with N addition and decreased precipitation. However, we speculated that prolonged N addition and decreased precipitation may induce an increase in the expression of inorganic transporters, especially for expression of NH_4^+ transporters N addition and decreased precipitation changed the root morphological traits and mycorrhizal colonization, but did not change the total N absorption amount among all tree species.

Author contribution statement

QW and TL designed the study, were awarded funding, supervised data collection and contributed to and edited manuscripts. MZ, GY and YX contributed the same for the whole manuscript preparation and design. QW, GY, YX, TL and MZ contributed the whole manuscript preparation and design and wrote the main manuscript text. QW, GY, YX, TL and MZ prepared all Figures, YX, FC, XZ, JW, JZ, GD, XZ, WS prepared field experiments, prepared tables and collected literatures. All authors reviewed the manuscript.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgments

We gratefully acknowledge Associate Professor Xingliang Xu from the Institute of Geographic Sciences and Natural Resource Research, Chinese Academy of Science for his advice about field experiment design and Professor Yunting Fang and Dr. Feifei Zhu from the Institute of Applied Ecology, Chinese Academy of Science for their comments and suggestions on an earlier draft of this manuscript. This research was supported by grants from the National Key Research and Development Program of China “Global Change and Response” (2016YFA0600800) and the National Natural Science Foundation of China (41773075, 41575137, 31370494, 31170421).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.09.166>.

References

- Arora, K., Srivastava, A., 2013. Nitrogen losses due to nitrification: plant based remedial prospects. *Int. J. Bioassays* 2 (07), 984–991.
- Averill, C., Finzi, A., 2011. Increasing plant use of organic nitrogen with elevation is reflected in nitrogen uptake rates and ecosystem $\delta^{15}\text{N}$. *Ecology* 92, 883–891.
- Bardgett, R.D., Streeter, T.C., Bol, R., 2003. Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. *Ecology* 84 (5), 1277–1287.
- Bassirirad, H., 2000. Kinetics of nutrient uptake by roots: responses to global change. *New Phytol.* 147 (1), 155–169.
- Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Glob. Chang. Biol.* 15, 808–824.
- Boudsocq, S., Niboyet, A., Lata, J.C., Raynaud, X., Loeuille, N., Mathieu, J., Blouin, M., Abbadie, L., Barot, S., 2012. Plant preference for ammonium versus nitrate: a neglected determinant of ecosystem functioning? *Am. Nat.* 180 (1), 60–69.
- Buljovic, Z., Engels, C., 2001. Nitrate uptake ability by maize roots during and after drought stress. *Plant Soil* 229 (1), 125–135.
- Comas, L.H., Mueller, K.E., Taylor, L.L., Midford, P.E., Callahan, H.S., Beerling, D.J., 2012. Evolutionary patterns and biogeochemical significance of angiosperm root traits. *Int. J. Plant Sci.* 173, 584–595.
- Eissenstat, D.M., Yanai, R.D., 1997. The ecology of root lifespan. *Adv. Ecol. Res.* 27, 1–60.
- Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* 10, 1135–1142.
- Emmett, B.A., Beier, C., Estiarte, M., Tietema, A., KristensenDylan, H.L., PeñuelasInger, W., Sowerby, S., 2004. The response of soil processes to climate change: results from manipulation studies of shrublands across an environmental gradient. *Ecosystems* 7, 625–637.
- Fitter, H., Stickland, T.R., 1992. Architectural analysis of plant root systems III: studies on plants under field conditions. *New Phytol.* 121 (2), 243–248.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320, 889–892.
- Geng, S., Chen, Z., Han, S., Wang, F., Zhang, J., 2017. Rainfall reduction amplifies the stimulatory effect of nitrogen addition on N_2O emissions from a temperate forest soil. *Sci. Rep.* 7.
- Gholamhoseini, M., Ghalavand, A., Dolatabadian, A., Jamshidi, E., Khodaei-Joghan, A., 2013. Effects of arbuscular mycorrhizal inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress. *Agric. Water Manag.* 117, 106–114.
- Glass, A.D., 2009. Nitrate uptake by plant roots. *Botany* 87 (7), 659–667.
- Grime, J.P., 2006. *Plant Strategies, Vegetation Processes, and Ecosystem Properties*. John Wiley & Sons.
- Gundersen, P., 1991. Nitrogen deposition and the forest nitrogen cycle: role of denitrification. *For. Ecol. Manag.* 44 (1), 15–28.
- Gutschick, V.P., 1981. Evolved strategies in nitrogen acquisition by plants. *Am. Nat.* 118 (5), 607–637.
- Hawkins, H.J., Johansen, A., George, E., 2000. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant Soil* 226 (2), 275–285.
- Inselsbacher, E., Näsholm, T., 2012. The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. *New Phytol.* 195 (2), 329–334.
- Kadam, N., Tamilselvan, A., Lawas, L.M.F., Quinones, C., Bahuguna, R., Thomson, M.J., Dingkuhn, M., Muthurajan, R., Struik, P., Yin, X., Jagadish, K., 2017. Genetic control of plasticity in root morphology and anatomy of rice in response to water-deficit. *Plant Physiol.* 00500.

- Kou, L., Guo, D.L., Yang, H., Gao, W.L., Li, S.G., 2015. Growth, morphological traits and mycorrhizal colonization of fine roots respond differently to nitrogen addition in a slash pine plantation in subtropical China. *Plant Soil* 391, 207–218.
- Larsen, K.S., Andresen, L.C., Beier, C., Jonasson, S., Albert, K.R., Ambus, P.E.R., Arndal, M.F., Carter, M.S., Christensen, S., Holmstrup, M., Ibrom, A., Kongstad, J., van der Linden, L., Maraldo, K., Michelsen, A., Mikkelsen, T.N., Pilegaard, K., Priemé, A., Ro-poulsen, H., Schmidt, I.K., Selsted, M.B., Stevnbak, K., 2011. Reduced N cycling in response to elevated CO₂, warming, and drought in a Danish heathland: synthesizing results of the CLIMATE project after two years of treatments. *Glob. Chang. Biol.* 17 (5), 1884–1899.
- LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89, 371–379.
- Leduc, S.D., Rothstein, D.E., 2010. Plant-available organic and mineral nitrogen shift in dominance with forest stand age. *Ecology* 91, 708–720.
- Li, W., Jin, C., Guan, D., Wang, Q., Wang, A., Yuan, F., Wu, J., 2015. The effects of simulated nitrogen deposition on plant root traits: a meta-analysis. *Soil Biol. Biochem.* 82, 112–118.
- Li, X., Rennenberg, H., Simon, J., 2015. Competition for nitrogen between *Fagus sylvatica* and *Acer pseudoplatanus* seedlings depends on soil nitrogen availability. *Front. Plant Sci.* 6 (April), 1–11.
- Lilleskov, E.A., Hobbie, E.A., Fahey, T.J., 2002. Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. *New Phytol.* 154, 219–231.
- Lipson, D., Näsholm, T., 2001. The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia* 128, 305–316.
- Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., Vitousek, P., Erisman, J.W., Goulding, K., Christie, P., Fangmeier, A., Zhang, F., 2013. Enhanced nitrogen deposition over China. *Nature* 494 (7438), 459–462.
- Liu, M., Li, C., Xu, X., Wanek, W., Jiang, N., Wang, H., Yang, X., 2017. Organic and inorganic nitrogen uptake by 21 dominant tree species in temperate and tropical forests. *Tree Physiol.* 1–12.
- Lü, C., Tian, H., 2007. Spatial and temporal patterns of nitrogen deposition in China: synthesis of observational data. *J. Geophys. Res.* 112, D22S05.
- McCormack, M.L., Dickie, I.A., Eissenstat, D.M., Fahey, T.J., Fernandez, C.W., Guo, D., Helmsaari, H., Hobbie, E.A., Iversen, C.M., Jackson, R.B., Leppälampi-Kujansuu, J., Norby, R.J., Phillips, R.P., Pregitzer, K.S., Pritchard, S.G., Rewald, B., Zadworny, M., 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytol.* 207 (3), 505–518.
- McKane, R.B., Johnson, L.C., Shaver, G.R., Nadelhoffer, K.J., Rastetter, E.B., Fry, B., Giblin, A.E., Kielland, K., Kwiatkowski, B.L., Laundre, J.A., Murray, G., 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature* 415 (6867), 68–71.
- Miller, A.E., Bowman, W.D., Suding, K.N., 2007. Plant uptake of inorganic and organic nitrogen: neighbor identity matters. *Ecology* 88 (7), 1832–1840.
- Näsholm, T., Kielland, K., Ganeteg, U., 2009. Uptake of organic nitrogen by plants. *New Phytol.* 182 (1), 31–48.
- Nordin, A., Höglberg, P., Näsholm, T., 2001. Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. *Oecologia* 129 (1), 125–132.
- Ostonen, I., Truu, M., Helmsaari, H.S., Lukac, M., Borken, W., Vanguelova, E., Godbold, D.L., Lohmus, K., Zang, U., Tedersoo, L., Preem, J., Rosenthal, K., Aosaar, J., Armolaitis, K., Frey, J., Kabral, N., Kukumägi, M., Leppälampi-Kujansuu, J., Lindroos, A., Merilä, P., Napa, Ü., Nöjd, P., Parts, K., Uri, V., Varik, M., Truu, J., 2017. Adaptive root foraging strategies along a boreal–temperate forest gradient. *New Phytol.* 215 (3), 977–991.
- Persson, J., Näsholm, T., 2001. Amino acid uptake: a widespread ability among boreal forest plants. *Ecol. Lett.* 4 (5), 434–438.
- Purugganan, M.D., Fuller, D.Q., 2009. The nature of selection during plant domestication. *Nature* 457 (7231), 843–848.
- Raab, T.K., Lipson, D.A., Monson, R.K., 1996. Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myosuroides*: implications for the alpine nitrogen cycle. *Oecologia* 108, 488–494.
- Rouphael, Y., Cardarelli, M., Schwarz, D., Franken, P., Colla, G., 2012. Effects of drought on nutrient uptake and assimilation in vegetable crops. In: Aroca, R. (Ed.), *Plant Responses to Drought Stress*. Springer, Berlin, Heidelberg, Germany, pp. 171–195.
- Sanaullah, M., Rumpel, C., Charrier, X., Chabbi, A., 2012. How does drought stress influence the decomposition of plant litter with contrasting quality in a grassland ecosystem? *Plant Soil* 352, 277–288.
- Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85, 591–602.
- Svennerstam, H., Ganeteg, U., Näsholm, T., 2008. Root uptake of cationic amino acids by *Arabidopsis* depends on functional expression of amino acid permease. *New Phytol.* 180, 620–630.
- Tobar, R.M., Azcón, R., Barea, J.M., 1994. Improved nitrogen uptake and transport from ¹⁵N-labelled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. *New Phytol.* 126, 119–122.
- Valverde-Barrantes, O.J., Smemo, K.A., Blackwood, C.B., 2015. Fine root morphology is phylogenetically structured, but nitrogen is related to the plant economics spectrum in temperate trees. *Funct. Ecol.* 29 (6), 796–807.
- Wang, Y., Cao, M.K., Tao, B., Ke rang, L.L., 2006. The characteristics of spatio-temporal patterns in precipitation in China under the background of global climate change. *Geogr. Res.* 25, 1031–1040.
- Wang, C., Han, S., Zhou, Y., Yan, C., Cheng, X., Zheng, X., Li, M.H., 2012. Responses of fine roots and soil N availability to short-term nitrogen fertilization in a broad-leaved Korean pine mixed forest in northeastern China. *PLoS One* 7 (3), e31042.
- Wang, G., Liu, F., Xue, S., 2017. Nitrogen addition enhanced water uptake by affecting fine root morphology and coarse root anatomy of Chinese pine seedlings. *Plant Soil* 418 (1–2), 177–189.
- Warren, C.R., 2009. Does nitrogen concentration affect relative uptake rates of nitrate, ammonium, and glycine? *J. Plant Nutr. Soil Sci.* 172, 224–229.
- Warren, C.R., Adams, P.R., 2007. Uptake of nitrate, ammonium and glycine by plants of Tasmanian wet eucalypt forests. *Tree Physiol.* 27, 413–419.
- Xu, X., Ouyang, H., Cao, G., Richter, A., Wanek, W., Kuzyakov, Y., 2011. Dominant plant species shift their nitrogen uptake patterns in response to nutrient enrichment caused by a fungal fairy in an alpine meadow. *Plant Soil* 341 (1–2), 495–504.
- Zarnetske, J.P., Haggerty, R., Wondzell, S.M., Bokil, V.A., González-Pinzón, R., 2012. Coupled transport and reaction kinetics control the nitrate source-sink function of hyporheic zones. *Water Resour. Res.* 48 (11).
- Zheng, J., Guo, R., Li, D., Zhang, J., Han, S., 2017. Nitrogen addition, drought and mixture effects on litter decomposition and nitrogen immobilization in a temperate forest. *Plant Soil* 1–15.